Preparation of competent cells

Electrocompetent E. coli cells

- 10% *E. coli* cells grown in 200 mL LB medium
- inoculate with 2-4 mL of an o/n culture, at 37 °C and 200 rpm until the OD_{600} reaches 0.3 - 0.5
- harvest cells by centrifugation (4 °C, 5 min, 3000 x g)
- wash twice with ice-cold 10% glycerol
- resuspend in ice-cold 10% glycerol to a final OD_{600} around 60 – 70
- aliquote in portions of 50 µL
- freeze in liquid nitrogen and store at -80 °C

$CaCl_2$ competent E. coli cells

- *E. coli* cells grown in 400 mL LB medium
- inoculate (1:100 ratio) with 4 mL of an o/n culture, at 37 °C and 200 rpm until OD_{600} reaches 0.6-0.7
- portion culture in 50 mL falcons and cool on ice
- harvest cells by centrifugation (4 °C, 10 min, 5000 x g)
- pellets are cooled on ice
- resuspend pellets in 10 mL of cold 100 mM $MgCl_2$ each for washing
- incubate on ice for 20-30 min
- unify two aliquots, centrifuge (4 °C, 10 min, 4000 rpm)
- resuspend pellets in 2 mL sterile ice-cold $CaCl_2$ solution (100 mM $CaCl_2$, 15% glycerol) each
- aliquote competent cells in portions of 50 µL, freeze in liquid nitrogen and store at -80 °C